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Phase I and II Cannabinoid Disposition in Blood and Plasma of Occasional and Frequent Smokers Following Controlled Smoked Cannabis

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BACKGROUND: Δ^9-Tetrahydrocannabinol (THC), 11-hydroxy-THC (11-OH-THC), and 11-nor-9-carboxy-THC (THCCOOH) have been reported in blood from frequent cannabis smokers for an extended time during abstinence. We compared THC, 11-OH-THC, THCCOOH, cannabidiol, cannabinol, THC-glucuronide, and 11-nor-9-carboxy-THC-glucuronide (THCCOO-glucuronide) blood and plasma disposition in frequent and occasional cannabis smokers.

METHODS: Frequent and occasional smokers resided on a closed research unit and smoked one 6.8% THC cannabis cigarette ad libitum. Blood and plasma cannabinoids were quantified on admission (approximately 19 h before), 1 h before, and up to 15 times (0.5–30 h) after smoking.

RESULTS: Cannabinoid blood and plasma concentrations were significantly higher in frequent smokers compared with occasional smokers at most time points for THC and 11-OH-THC and at all time points for THCCOOH and THCCOO-glucuronide. Cannabidiol, cannabinol, and THC-glucuronide were not significantly different at any time point. Overall blood and plasma cannabinoid concentrations were significantly higher in frequent smokers for THC, 11-OH-THC, THCCOOH, and THCCOO-glucuronide, with and without accounting for baseline concentrations. For blood THC >5 μg/L, median (range) time of last detection was 3.5 h (1.1–>30 h) in frequent smokers and 1.0 h (0–2.1 h) in 11 occasional smokers; 2 individuals had no samples with THC >5 μg/L.

CONCLUSIONS: Cannabis smoking history plays a major role in cannabinoid detection. These differences may impact clinical and impaired driving drug detection. The presence of cannabidiol, cannabinol, or THC-glucuronide indicates recent use, but their absence does not exclude it.

Cannabis is the most commonly abused illicit drug worldwide, with 2.6%–5.0% (119–224 million people) of 15- to 64-year-olds consuming cannabis at least once in 2010 *(1)*. In 2012, 11%, 28%, and 36% of American eighth, 10th, and 12th graders smoked cannabis at least once, respectively, with 1.1%, 3.5% and 6.5% smoking the drug daily or near daily *(2)*. Δ^9-Tetrahydrocannabinol (THC),[3] the main psychoactive ingredient in cannabis, was the most prevalent illicit drug detected in injured drivers in Victoria, Australia (9.8%) *(3)*. Cannabinoids were also found in 8.6% of nighttime drivers' blood and/or oral fluid in the 2007 US Roadside Survey *(4)*. Thus, cannabis continues to be a drug of concern.

THC appears in plasma immediately after the first puff and peaks before the last puff on a cannabis cigarette *(5)*. THC is rapidly metabolized to the active 11-hydroxy-THC (11-OH-THC) by cytochrome P450 2C9, 2C19, and 3A4, with concentrations peaking approximately 13 min after smoking and reaching 10% of THC concentrations *(6)*. 11-OH-THC is further oxidized to the inactive 11-nor-9-carboxy-THC (THCCOOH); THCCOOH plasma concentrations slowly increase over the first hour after smoking and plateau after 2–4 h. Characterization of phase II cannabinoid metabolite disposition remains incomplete *(7–9)*, although in vitro studies indicate that cannabinoids are primarily glucuronidated, with possible minor sulfa-

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[3] Nonstandard abbreviations: THC, Δ^9-tetrahydrocannabinol; 11-OH-THC, 11-hydroxy-THC; THCCOOH, 11-nor-9-carboxy-THC; UGT, UDP-glucuronosyltransferase; CBD, cannabidiol; CBN, cannabinol; C_{max}, maximal concentration; THCCOO-glucuronide, 11-nor-9-carboxy-THC-glucuronide; AUC, area under the curve; LC-MS/MS, liquid chromatography–tandem mass spectrometry; NIDA, National Institute on Drug Abuse; LOQ, limit of quantification; t_{max}, time at maximal concentration; t_{last}, time of last positive result.

tion *(10)*. THC and 11-OH-THC are conjugated through UDP-glucuronosyltransferase (UGT) 1A9 and 1A10, and THCCOOH is conjugated by UGT 1A3, but also 1A1, 1A4, 1A6, and 1A7 *(10)*.

Because of their high lipophilicity, which results in extensive storage and prolonged release from adipose tissue, as well as enterohepatic recirculation, cannabinoids remain in plasma for extended periods of abstinence in chronic daily smokers. Recent work by Bergamaschi et al. documented free THC detection for 30 days, free 11-OH-THC for 3 days, and free THCCOOH detection for at least 33 days in plasma from 1 chronic daily smoker during abstinence *(11)*. With this long detection window, blood markers of recent cannabis intake are required when documenting impaired driving, accident responsibility, and new use. We recently demonstrated that cannabidiol (CBD), cannabinol (CBN), and THC-glucuronide in plasma were inclusionary markers of recent ($\leq$2 h) intake in chronic frequent smokers *(7)*.

Few studies have directly compared cannabinoid pharmacokinetics in occasional and frequent smokers. Toennes et al. *(12)* demonstrated that THC and THCCOOH maximal concentrations (C_{max}) and areas under the curves from smoking to 8 h (AUC_{0-8}) were significantly higher in frequent smokers compared to occasional smokers; however, after adjusting for baseline concentrations, THC, but not THCCOOH, C_{max} and AUC were significantly higher in frequent smokers, possibly due to altered smoking topography. In addition, distribution and elimination patterns were comparable between the 2 groups. Kelly and Jones *(8)* documented significantly higher THCCOOH and 11-nor-9-carboxy-THC-glucuronide (THCCOO-glucuronide) (THCCOO-glucuronide calculated from total minus free THCCOOH) in frequent smokers compared with occasional smokers after intravenous THC administration; there were no group differences in THC. Skopp and Pötsch *(9)* evaluated frequent, moderate, and occasional smokers and suggested higher THC, 11-OH-THC, THCCOOH, and THCCOO-glucuronide concentrations in the 16 frequent smokers' samples collected 24–48 h after admission to a detoxification center, although no statistical evaluation was conducted.

In this study, we evaluated THC, 11-OH-THC, THCCOOH, CBD, CBN, THC-glucuronide, and THCCOO-glucuronide in 14 frequent and 11 occasional smokers following a single smoked 6.8% THC cigarette to investigate group differences. We directly and simultaneously (without hydrolysis) monitored cannabinoid phase I and II metabolites in blood and plasma for up to 30 h after controlled smoking by liquid chromatography–tandem mass spectrometry (LC-MS/MS). Our goal was to provide a comprehensive cannabinoid metabolic profile in humans, thus providing important information on cannabinoid metabolism and documenting windows of drug detection in frequent and occasional smokers.

Methods

PARTICIPANTS

Healthy subjects provided written informed consent to participate in this National Institute on Drug Abuse (NIDA) Intramural Research Program Institutional Review Board–approved study. Individuals were recruited by radio and newspaper advertisements, flyers, and participant referrals. Participants received a comprehensive medical and psychological evaluation to verify compliance with eligibility criteria. Inclusion criteria were ages 18–45 years and self-reported mean smoked cannabis frequency of less than twice per week (occasional smoker) or at least 4 times per week (frequent smoker) in the past 3 months. Current history of cannabis use in frequent smokers was confirmed by a positive urine cannabinoid test; occasional smokers were not required to be positive. Exclusion criteria included breastfeeding or pregnant women, current medical condition or history of neurological illness, history of a clinically significant adverse event associated with cannabis intoxication, donation of >450 mL blood within 30 days of drug administration, presence of clinically significant anemia, increased systolic or diastolic blood pressure or heart rate >100 bpm after 5-min rest, clinically significant electrocardiogram abnormality, or interested in drug abuse treatment within 60 days of screening. Pregnancy tests were administered at screening and on study admission to women with reproductive potential.

STUDY DESIGN

Participants entered the secure research unit approximately 19 h before smoking to preclude intoxication at the time of cannabis dosing. Cannabis cigarettes were obtained through the NIDA Chemistry and Physiological Systems Research Branch. Participants smoked 1 [mean (SD)] 6.8% (0.2%) THC (54 mg), 0.25% (0.08%) CBD (2 mg), and 0.21% (0.02%) CBN (1.6 mg) cannabis cigarette ad libitum for up to 10 min. We collected venous blood through an indwelling peripheral intravenous catheter into sodium heparin plastic Vacutainer blood tubes. Samples were collected on admission, 1 h before, and 0.5, 1, 2, 3, 4, 5, 6, 8, 10.5, 13.5, 21, 24, 26, 28, and 30 h after the start of smoking and were kept on ice. Blood collected for plasma was centrifuged (1600*g*, 10 min, 4 °C). Blood and plasma were aliquoted into 3.6-mL polypropylene Nunc cryotubes (Thomas Scientific), stored frozen at −20 °C, and analyzed within 3 months, a duration within which ana-

lytes are stable *(13)*. We collected samples only while participants were on the secure residential unit through study discharge, which occurred no earlier than 6 h after cannabis smoking.

BLOOD AND PLASMA ANALYSIS

We quantified cannabinoids by a previously validated LC-MS/MS method *(14)*. Briefly, 0.5 mL whole blood or plasma was deproteinized with acetonitrile and the supernatants diluted before solid-phase extraction. The eluent was evaporated, reconstituted in mobile phase, centrifuged, and injected onto the LC-MS/MS instrument. Linear ranges were 1–100 μg/L for THC, 11-OH-THC, THCCOOH, CBD, and CBN; 0.5–50 μg/L for THC-glucuronide; and 5–500 μg/L for THCCOO-glucuronide [the lowest number being the limit of quantification (LOQ)]. Interassay (n = 20) analytical bias and imprecision were 93.8%–113.1% and 4.9%–10.4%, respectively.

STATISTICAL ANALYSIS

We performed noncompartmental pharmacokinetic analyses with Phoenix WinNonlin® 6.3 for Windows (Pharsight Software) for analytes with sufficient (>5) positive samples (THC, 11-OH-THC, THCCOOH, and THCCOO-glucuronide). Group differences, including demographics and drug use, highest observed concentrations (C_{max}), postdose C_{max}, postdose time at C_{max} (t_{max}), time of last positive result (t_{last}), AUC_{0-30} (AUC from 0 to 30 h postdose), $AUC_{>baseline}$ [AUC of time above baseline concentration (1 h before smoking)], and concentrations at each time point were compared with Mann–Whitney (exact test) by use of SPSS® for Windows version 20 (IBM). Bonferroni correction was applied to comparisons of concentrations at each time point to control for multiple comparisons, yielding a 2-tailed α level of $P < 0.003$ (0.05/17 number of time points). We determined mean, SD, and 95% confidence interval for baseline concentrations and C_{max} for frequent and occasional smokers with 1-sample *t*-test in SPSS. Any confidence interval below 0 was constrained to exclude negative concentrations. Fold change between frequent and occasional smokers' C_{max} were evaluated when ≥5 participants from each group were positive for a given analyte by dividing frequent smokers' minimum concentration with occasional smokers' maximum concentration (low end of fold change) and by dividing frequent smokers' maximum concentration with occasional smokers' minimum concentration (high end of fold change).

We analyzed overall group effects in concentration (log transformed to yield normally distributed concentrations) and THCCOO-glucuronide:THCCOOH ratios with general linear mixed models in SPSS, with time as a covariate and with and without admission concentration as a covariate (in the case of concentrations); significance was attributed at $P < 0.05$.

Results

HUMAN PARTICIPANTS

Fourteen frequent and 11 occasional smokers (18 men, 7 women), ages 19–41 years, participated in the study (Table 1). Frequent smokers were significantly younger, smoked for fewer lifetime years, and smoked more recently in the prior 14 days and more frequently compared to occasional smokers. Two participants (M and N) were originally classified as occasional users by self-report, but were reclassified as frequent smokers because their baseline and all postsmoking THC and metabolite concentrations were consistent with published blood *(7)*, oral fluid *(15)*, and urine *(16)* cannabinoid concentrations from frequent smokers. Participants B, J, T, and Y withdrew from the study early, after the 26-, 10.5-, 24-, and 6-h sample collections, respectively. Additional blood and plasma samples were missed for participant G at 0.5 and 2 h, I at 10.5 h, L at 13.5 h, and M (plasma only) at 13.5 h, due to catheter blockage.

BLOOD AND PLASMA ANALYSIS

When the 402 blood (226 frequent, 176 occasional) and 401 plasma (225 frequent, 176 occasional) samples were quantified for cannabinoids (Fig. 1; Supplemental Figs. 1 and 2, which accompany the online version of this article at http://www.clinchem.org/content/vol60/issue4), cannabinoid blood and plasma concentrations were found to be significantly higher in frequent smokers compared to occasional smokers at most time points for THC and 11-OH-THC and at all time points for THCCOOH and THCCOO-glucuronide (Fig. 1, online Supplemental Figs. 1 and 2). CBD, CBN, and THC-glucuronide were not significantly different at any time point. THC C_{max} values were 0.4- to 12.1-fold higher in frequent smokers' blood and 0.3- to 12.9-fold higher in plasma compared with occasional smokers; THCCOOH C_{max} values were 1.1- to 18.4-fold higher in blood and 0.9- to 18.3-fold higher in plasma of frequent smokers (see online Supplemental Table 1).

Overall blood and plasma cannabinoid concentrations were significantly higher in frequent smokers for THC, 11-OH-THC, THCCOOH, and THCCOO-glucuronide, with and without accounting for baseline concentrations (Table 2). The effect of baseline concentration was not evaluated for CBD, CBN, and THC-glucuronide, as all baseline samples were negative.

Pharmacokinetic parameters for each analyte are presented in Tables 3 and 4. THCCOOH t_{last} group differences could not be evaluated because all participants were still positive at 30 h postdose.

Table 1. Demographic characteristics and cannabis smoking histories for 14 frequent and 11 occasional smokers.

Participant	Race and ethnicity	Sex	Age at admission	Body mass index[a]	Age at first use[a]	Lifetime years smoked[a]	Time between last use and admission	Number of days used in last 14[b]	Average joint or joint equivalent[b]
Frequent smoker									
A	B[c]	M	29.6	27.6	12	17.6	7.4 h	11	4/day
B	B	M	19.4	22.6	15	4.4	4.3 h	13	5/day
C	B	M	22.6	31.4	14	8.6	5.1 h	12	3/day
D	W	M	25.5	23.0	13	12.5	3.9 h	14	20/day
E	B	F	19.9	32.4	11	8.9	2.6 h	14	3.5/day
F	B	M	24.2	27.4	13	11.2	23.2 h	12	1.5/day
G	W	F	22.9	24.8	16	6.9	17.2 h	14	6/day
H	B	M	37.3	23.0	25	12.3	1.6 h	14	3/day
I	B	F	27.6	35.4	18	9.6	2.4 h	14	4/day
J	B	F	26.9	20.4	14	12.9	3.8 h	14	21/day
K	B	M	23.4	24.3	19	4.4	1.2 h	14	6/day
L	B	M	28.7	28.1	14	14.7	9.5 h	14	6/day
M	B	M	28.0	19.4	14	14.0	67.4 h[d]	2[d]	2/month[d]
N	B	M	23.8	30.7	14	9.8	273 h[d]	1[d]	4/month[d]
Mean			25.7	26.4	15.1	10.6		13.3	
SD			4.6	4.8	3.5	3.8		1.1	
Median			24.8	26.1	14.0	10.5	4.1 h	14.0	4.5/day
Occasional smoker									
O	W	M	25.6	29.4	16	9.6	16 days	0	2/month
P	W	M	25.4	23.7	13	12.4	31 days	0	2/month
Q	W	M	23.7	24.1	16	7.7	10 days	2	7/month
R	B	M	38.2	21.0	19	19.2	2 days	2	2/month
S	M	M	41.3	22.0	16	25.3	7 days	5	10/month[e]
T	U	F	34.9	31.7	13	21.9	9 days	1	2/month
U	B	F	36.5	47.8	18	18.5	2 days	2	4/month
V	M, H	M	22.5	25.2	13	9.5	86 days	0	6/month
W	W	F	34.2	26.6	14	20.2	3 days	1	0.25/month
X	B, U	M	31.7	21.8	16	15.7	18 days	0	8/month
Y	B	M	31.9	22.6	15	16.9	68 days	0	2/month
Mean			31.4	26.9	15.4	16.1		1.2	
SD			6.3	7.7	2.0	5.7		1.5	
Median			31.9[f]	24.1	16.0	16.9[f]	10 days[f]	1.0[f]	2/month[f]

[a] Data collected at study admission.
[b] Data collected prior to smoking.
[c] B, black or African American; W, white; M, mixed; U, unknown; H, Hispanic or Latino.
[d] Self-reported data not consistent with biological sample concentrations. Data excluded from mean and median.
[e] Self-reported average use at screening of 0.5 joints, 3–4 times per month.
[f] Significant difference between groups ($P < 0.05$).

DETECTION RATES

THCCOOH had the highest detection rate, followed by THCCOO-glucuronide, THC, and 11-OH-THC (Fig. 2, online Supplemental Figs. 3 and 4). When present, CBD, CBN, and THC-glucuronide were detected for only 0.5–4 h. For blood THC >5 μg/L, median (range) time of last detection was 3.5 h (1.1 to >30 h) in frequent smokers and 1.0 h (0–2.1 h) in occasional smokers (Mann–Whitney $U = 9.5$, $z = -3.698$, $P < 0.001$) (Fig. 2, online Supplemental Figs. 3 and 4); 2 occasional

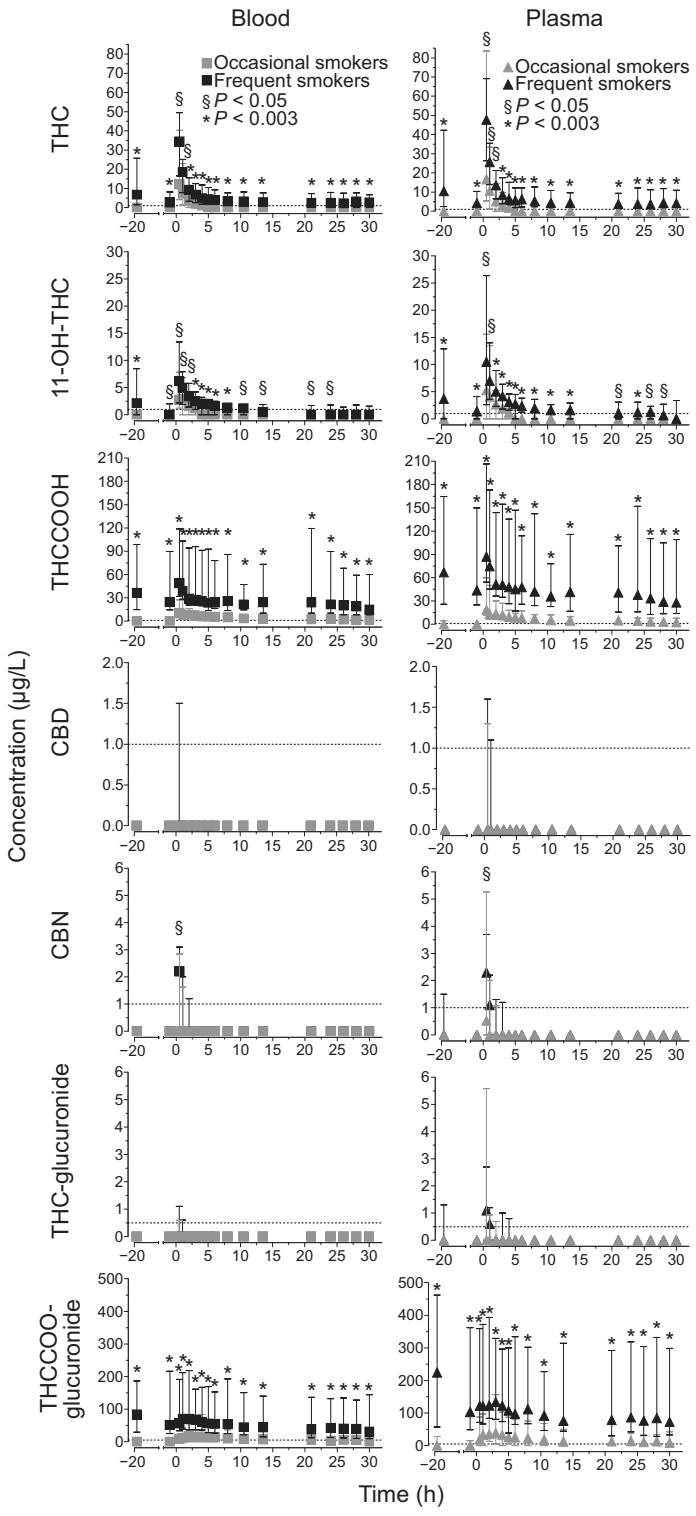

Fig. 1. Median (range) blood and plasma concentrations in 14 frequent and 11 occasional smokers following smoking of a 6.8% THC cannabis cigarette.

Dashed line is LOQ.

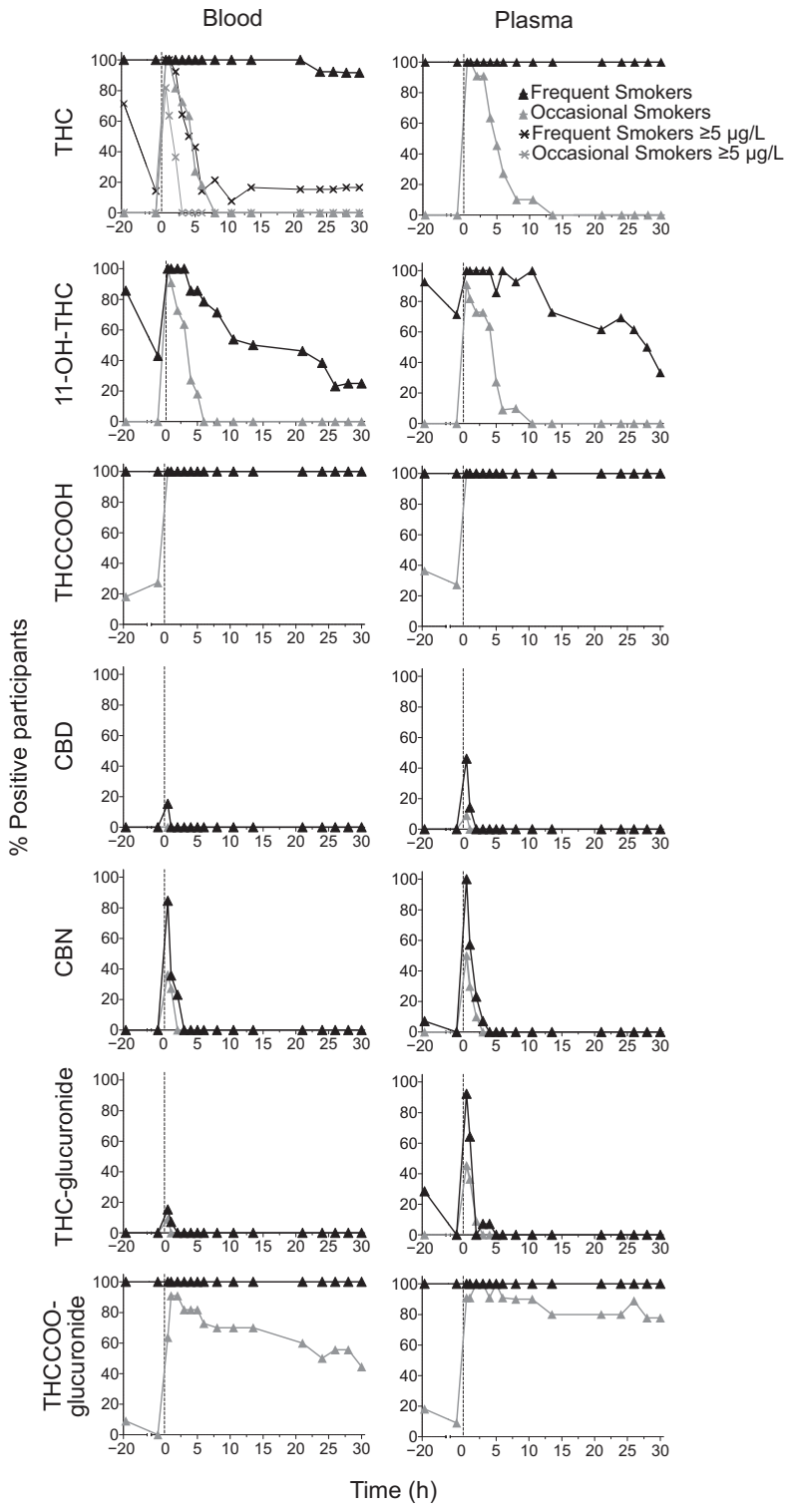

Fig. 2. Cannabinoid blood and plasma detection rates in 14 frequent and 11 occasional smokers following smoking of a 6.8% THC cannabis cigarette.

Dashed line is time of smoking.

Table 2. Overall effect of group, time, and baseline concentration on cannabinoid blood and plasma concentrations in 14 frequent and 11 occasional smokers following smoking of a 6.8% THC cannabis cigarette.[a]

		Blood				Plasma			
		Not accounting for baseline		Accounting for baseline		Not accounting for baseline		Accounting for baseline	
Analyte	Effect	*t*	*P*	*t*	*P*	*t*	*P*	*t*	*P*
THC	Group	34.1	**<0.001**	24.8	**<0.001**	39.6	**<0.001**	34.5	**<0.001**
	Time	−6.7	**<0.001**	−9.0	**<0.001**	−6.6	**<0.001**	−5.1	**<0.001**
	Baseline concentration	N/A	N/A	25.1	**<0.001**	N/A	N/A	31.2	**<0.001**
11-OH-THC	Group	11.0	**<0.001**	4.3	**<0.001**	22.5	**<0.001**	11.7	**<0.001**
	Time	−9.7	**<0.001**	−10.3	**<0.001**	−10.7	**<0.001**	−11.8	**<0.001**
	Baseline concentration	N/A	N/A	12.4	**<0.001**	N/A	N/A	14.0	**<0.001**
THCCOOH	Group	21.9	**<0.001**	17.3	**<0.001**	34.6	**<0.001**	21.1	**<0.001**
	Time	−7.8	**<0.001**	−13.3	**<0.001**	−10.7	**<0.001**	−14.3	**<0.001**
	Baseline concentration	N/A	N/A	27.3	**<0.001**	N/A	N/A	18.0	**<0.001**
CBD	Group	0.002	0.999	0.002	0.999	0.003	0.998	0.003	0.998
	Time	−0.001	0.999	−0.001	0.999	−0.002	0.998	−0.002	0.998
	Baseline concentration	N/A	N/A			N/A	N/A		
CBN	Group	0.002	0.998	0.002	0.998	0.005	0.996	0.005	0.996
	Time	−0.003	0.998	−0.003	0.998	−0.017	0.987	−0.017	0.987
	Baseline concentration	N/A	N/A			N/A	N/A		
THC-glucuronide	Group	0.004	0.997	0.004	0.997	0.005	0.996	0.005	0.996
	Time	−0.006	0.996	−0.006	0.996	0.030	0.976	0.030	0.976
	Baseline concentration	N/A	N/A			N/A	N/A		
THCCOO-glucuronide	Group	15.4	**<0.001**	13.3	**<0.001**	20.6	**<0.001**	9.9	**<0.001**
	Time	−4.4	**<0.001**	−6.0	**<0.001**	−5.8	**<0.001**	−6.5	**<0.001**
	Baseline concentration	N/A	N/A	15.6	**<0.001**	N/A	N/A	8.1	**<0.001**

[a] Values in bold are statistically significant.

smokers were never positive, and 2 frequent smokers (I and K) were >5 μg/L at baseline and 30 h.

BLOOD-TO-PLASMA RATIOS

Overall median (range) blood-to-plasma ratios were 0.68 (0.31–1.1), 0.63 (0.38–1.1), 0.59 (0.41–1.2), 0.84 (0.47–1.3), and 0.47 (0.24–1.1) for THC, 11-OH-THC, THCCOOH, CBN, and THCCOO-glucuronide. Too few positive samples occurred for accurate CBD and THC-glucuronide ratio calculation.

THCCOO-GLUCURONIDE:THCCOOH RATIOS

Occasional smokers had higher THCCOO-glucuronide:THCCOOH ratios in blood and plasma; median ratios decreased immediately after smoking (see online Supplemental Fig. 5). However, high variability was observed between groups and over time. There was a significant main effect of time on THCCOO-glucuronide:THCCOOH ratios in blood ($t = -0.6$, $P = 0.538$ for group and $t = 3.5$, $P < 0.01$ for time) and plasma ($t = -1.8$, $P = 0.075$ for group and $t = 4.8$ and $P < 0.001$ for time), but no significant main group effect.

Discussion

We recently documented phase I and II cannabinoids detection in blood and plasma following smoked cannabis, with CBD, CBN, and THC-glucuronide proposed as potential inclusionary markers of recent use; however, only chronic frequent cannabis smokers were recruited *(7)*. In this study, we evaluated THC, 11-OH-THC, THCCOOH, CBD, CBN, THC-glucuronide, and THCCOO-glucuronide in frequent and occasional smokers for up to 30 h after smoking to evaluate these as markers of recent use. We observed frequent cannabis smoker cannabinoid concentrations similar to those previously reported *(7)*.

Table 3. Median (range) blood pharmacokinetic group comparison in 14 frequent and 11 occasional smokers following smoking of a 6.8% cannabis cigarette.[a]

Analyte and parameter	Frequent smoker	Occasional smoker	Mann–Whitney *U*	*z*	*P*
THC					
Positive participants, %	100	100			
C_{max}, μg/L	34.4 (16.5–49.5)	12.1 (4.1–40.3)	26.0	−2.636	**<0.01**
Postdose C_{max}, μg/L	34.4 (16.5–49.5)	12.1 (4.1–40.3)	26.0	−2.636	**<0.01**
Postdose t_{max}, h	0.5 (0.5–0.6)	0.5 (0.5–0.6)	63.5	−0.471	0.655
t_{last}, h	>30 (24.0–>30)	4.0 (1.0–6.0)	0	−4.304	**<0.001**
$AUC_{0–30}$	104 (51.2–236.1)	18.2 (3.1–57.3)	0	−4.215	**<0.001**
$AUC_{>baseline}$	45.2 (25.4–60.2)	18.2 (3.1–57.3)	30.0	−2.573	**<0.01**
11-OH-THC					
Positive participants, %	100	100			
C_{max}, μg/L	6.7 (2.2–13.4)	2.9 (1.6–7.9)	20.0	−2.984	**<0.01**
Postdose C_{max}, μg/L	6.2 (2.2–13.4)	2.9 (1.6–7.9)	22.0	−2.868	**<0.01**
Postdose t_{max}, h	0.5 (0.5–0.6)	0.5 (0.5–1.0)	63.5	−0.470	0.656
t_{last}, h	12.0 (3.1–>30)	3.0 (1.0–5.0)	9.0	−3.728	**<0.001**
$AUC_{0–30}$	32.5 (5.3–65.8)	7.1 (1.7–16.7)	14.0	−3.280	**<0.001**
$AUC_{>baseline}$	19.5 (5.3–41.6)	6.1 (0.6–16.7)	22.0	−3.011	**<0.01**
THCCOOH					
Positive participants, %	100	100			
C_{max}, μg/L	52.8 (31.9–119)	10.4 (6.5–27.4)	0	−4.142	**<0.001**
Postdose C_{max}, μg/L	48.7 (31.9–119)	10.4 (6.5–27.4)	0	−4.142	**<0.001**
Postdose t_{max}, h	0.5 (0.5–21.0)	0.5 (0.5–1.0)	68.5	−0.175	0.875
t_{last}, h	>30	>30	N/A	N/A	N/A
$AUC_{0–30}$	689.2 (473–2591)	113.4 (63.0–216)	0	−4.215	**<0.001**
$AUC_{>baseline}$	54.7 (12.3–212)	86.9 (43.7–182)	42.0	−1.916	0.058
CBD					
Positive participants, %	15.4	0			
Postdose C_{max}, μg/L	0 (0–1.5)	0	60.5	−1.329	0.482
Postdose t_{max}, h	0 (0–0.5)	0	60.5	−1.329	0.482
t_{last}, h	0 (0–0.5)	0	60.5	−1.329	0.482
CBN					
Positive participants, %	84.6	36.4			
Postdose C_{max}, μg/L	2.2 (0–3.1)	0 (0–2.8)	38.0	−1.994	**<0.05**
Postdose t_{max}, h	0.5 (0–0.6)	0 (0–0.6)	41.0	−1.824	0.070
t_{last}, h	0.6 (0–2.1)	0 (0–1.1)	38.0	−1.997	**<0.05**
THC-glucuronide					
Positive participants, %	15.4	9.1			
Postdose C_{max}, μg/L	0 (0–1.1)	0 (0–0.6)	66.0	−0.554	0.717
Postdose t_{max}, h	0 (0–0.5)	0 (0–0.6)	68.0	−0.353	1.000
t_{last}, h	0 (0–0.5)	0 (0–0.6)	68.0	−0.353	1.000
THCCOO-glucuronide					
Positive participants, %	100	90.9			
C_{max}, μg/L	84.1 (52.2–218)	16.2 (0–83.4)	8.0	−3.777	**<0.001**

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Table 3. Median (range) blood pharmacokinetic group comparison in 14 frequent and 11 occasional smokers following smoking of a 6.8% cannabis cigarette.[a] *(Continued from page 638)*

Analyte and parameter	Frequent smoker	Occasional smoker	Mann–Whitney *U*	*z*	*P*
Postdose C_{max}, μg/L	74.3 (40.4–218)	16.2 (0 to −83.4)	12.0	−3.558	**<0.001**
Postdose t_{max}, h	2.1 (0.5–6.0)	3.0 (0–5.0)	49.5	−1.508	0.137
t_{last}, h	>30	27.0 (0–>30)	24.0	−3.026	**<0.01**
$AUC_{0–30}$	1342 (597–4264)	382 (150–762)	3.0	−3.617	**<0.001**
$AUC_{>baseline}$	70.1 (0.37–358)	212 (11.2–762)	38.0	−1.874	0.064

[a] Values in bold are statistically significant.

We applied conservative Bonferroni corrections accounting for multiple comparisons for evaluating group differences. This reduced the odds of type I statistical errors; however, it may have limited statistical group differences. We applied $P < 0.003$ significance threshold, but also indicated group differences of $P < 0.05$ for informational purposes (Fig. 1).

THC and 11-OH-THC concentrations were higher in frequent smokers, except immediately after smoking, in plasma and blood (THC and 11-OH-THC) and after approximately 10 h (11-OH-THC only). Large variation in smoking topography may explain the lack of significant group differences in the first 2 h; in fact, in plasma, an occasional smoker (participant R) demonstrated the highest THC C_{max}. After 2 h, THC and 11-OH-THC concentrations were significantly higher in frequent smokers. In occasional smokers, THC and 11-OH-THC were positive for <10.5 h. Blood and plasma THC and 11-OH-THC concentrations also dropped rapidly in frequent smokers, but had significantly later t_{last} and much longer detection windows due to residual blood THC from previous cannabis smoking *(11)*. At 6 h, THC and 11-OH-THC concentrations were similar to those reported by Schwope et al. *(7)*.

Blood THC >5 μg/L detection rates were evaluated because this concentration is currently the per se limit for driving under the influence of cannabis in Washington state, 1 of the 2 states that has legalized recreational cannabis in the US. At this cutoff, 2 occasional smokers were never positive, although they were most likely positive before the first blood collection at 0.5 h; 0.5-h THC blood concentrations were 4.8 and 4.1 μg/L. By 2 h, all occasional smokers were <5 μg/L. Therefore, many participants would not have been detected during THC's approximately 6-h acute psychomotor impairment window *(17)*. Previous research documented significant impairment on critical and divided attention in occasional smokers *(18)*. Hence, impaired occasional drivers could be overlooked with a 5-μg/L THC cutoff. In frequent smokers, 16.7% of participants were positive for >30 h at 5 μg/L. There is evidence supporting continued psychomotor impairment after 3 weeks of abstinence in chronic frequent smokers *(19)*, suggesting that driving ability is impaired at the time of these low blood THC concentrations. Hence, long windows of detection may be necessary to document residual impairment. More work is needed to fully correlate blood concentrations with impairment levels in frequent and occasional smokers.

Median THC and 11-OH-THC t_{max} were 0.5 h, the first collection time. The true t_{max} (and thus C_{max}) were not captured in this study because THC peaks before the last puff on a cannabis cigarette *(5)*. One participant had an observed THC t_{max} at 1 h. Similarly, a 1-h t_{max} was observed for 11-OH-THC, CBD, and CBN for this participant.

THC concentrations and 11-OH-THC $AUC_{0–30}$ in blood and plasma were significantly higher in frequent smokers, which is unsurprising given THC's highly lipophilic nature and extended excretion in chronic frequent smokers. Similarly, overall THC and 11-OH-THC concentrations were significantly higher in frequent smokers. After correcting for the baseline concentration ($AUC_{>baseline}$ and time-corrected overall concentrations), THC and 11-OH-THC $AUC_{0–30}$ remained significantly higher in frequent smokers compared to occasional smokers. The higher $AUC_{0–30}$ could result from frequent smokers inhaling significantly more cannabis smoke owing to tolerance to cannabis' subjective effects. Kelly and Jones *(8)* documented slightly higher but overlapping THC $AUC_{0–12\ days}$ in frequent smokers compared to occasional smokers following intravenous THC. Toennes et al. *(12)* documented higher THC and 11-OH-THC $AUC_{0–8}$ and C_{max} in frequent smokers than in occasional smokers following a paced-smoking procedure, even after correcting for initial THC concentration; this was attributed to better smoking efficiency in frequent smokers. Lee et al. *(20)* previously reported that chronic fre-

Table 4. Median (range) plasma pharmacokinetic group comparison in 14 frequent and 11 occasional smokers following smoking of a 6.8% THC cannabis cigarette.[a]

Analyte and parameter	Frequent smoker	Occasional smoker	Mann–Whitney *U*	*z*	*P*
THC					
Positive participants, %	100	100			
C_{max}, μg/L	47.7 (26.3–69.1)	16.7 (5.4–83.6)	32.0	−2.289	**<0.05**
Postdose C_{max}, μg/L	47.7 (26.3–69.1)	16.7 (5.4–83.6)	32.0	−2.289	**<0.05**
Postdose t_{max}, h	0.5 (0.4–1.1)	0.5	63.5	−0.470	0.655
t_{last}, h	>30	4.0 (1.0–10.5)	0	−4.320	**<0.001**
$AUC_{0–30}$	178 (109–374)	29.1 (3.6–107)	3.0	−4.051	**<0.001**
$AUC_{>baseline}$	61.6 (38.0–118)	29.1 (3.6–107)	33.0	−2.409	**<0.05**
11-OH-THC					
Positive participants, %	100	90.9			
C_{max}, μg/L	10.8 (4.0–26.4)	5.3 (0–15.6)	25.0	−2.694	**<0.01**
Postdose C_{max}, μg/L	10.5 (3.6–26.4)	5.3 (0–15.6)	27.0	−2.578	**<0.01**
Postdose t_{max}, h	0.5 (0.4–1.1)	0.6 (0–1.0)	48.5	−1.340	0.189
t_{last}, h	27.1 (10.5–>30)	4.0 (0–8.0)	0	−4.074	**<0.001**
$AUC_{0–30}$	65.0 (19.8–106)	11.4 (0.9–36.0)	4.0	−3.865	**<0.001**
$AUC_{>baseline}$	22.9 (10.2–50.5)	11.4 (0.9–36.0)	23.0	−2.752	**<0.01**
THCCOOH					
Positive participants, %	100	100			
C_{max}, μg/L	96.5 (54.2–207)	18.6 (11.3–59.7)	2.0	−4.027	**<0.001**
Postdose C_{max}, μg/L	87.0 (54.2–207)	18.6 (11.3–59.7)	3.0	−3.969	**<0.001**
Postdose t_{max}, h	0.5 (0.4–1.1)	0.5	63.5	−0.470	0.655
t_{last}, h	>30	>30	N/A	N/A	N/A
$AUC_{0–30}$	1231 (759–3724)	203 (124–346)	0	−4.215	**<0.001**
$AUC_{>baseline}$	78.7 (18.5–308)	140 (84.8–344)	28.0	−2.682	**<0.01**
CBD					
Positive participants, %	53.8	9.1			
Postdose C_{max}, μg/L	1.1 (0–1.6)	0 (0–1.3)	40.0	−2.175	**<0.05**
Postdose t_{max}, h	0.5 (0–1.1)	0 (0–0.5)	40.0	−2.177	**<0.05**
t_{last}, h	0.5 (0–1.1)	0 (0–0.5)	39.0	−2.246	**<0.05**
CBN					
Positive participants, %	100	50.0			
Postdose C_{max}, μg/L	2.3 (1.0–3.7)	0.5 (0–5.3)	30.0	−2.181	**<0.05**
Postdose t_{max}, h	0.5 (0.4–1.1)	0.2 (0–0.5)	37.0	−1.757	0.080
t_{last}, h	1.0 (0.4–3.0)	0.3 (0–2.1)	34.5	−1.903	0.058
THC-glucuronide					
Positive participants, %	92.3	45.5			
Postdose C_{max}, μg/L	1.1 (0–2.7)	0 (0–5.6)	48.5	−1.349	0.185
Postdose t_{max}, h	0.5 (0–0.6)	0 (0–0.5)	52.0	−1.150	0.263
t_{last}, h	1.0 (0–4.0)	0 (0–2.1)	47.5	−1.410	0.166
THCCOO-glucuronide					
Positive participants, %	100	100			
C_{max}, μg/L	228 (97.9–462)	38.4 (15.6–156)	7.0	−3.832	**<0.001**

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Table 4. Median (range) plasma pharmacokinetic group comparison in 14 frequent and 11 occasional smokers following smoking of a 6.8% THC cannabis cigarette.[a] *(Continued from page 640)*

Analyte and parameter	Frequent smoker	Occasional smoker	Mann–Whitney *U*	*z*	*P*
Postdose C_{max}, μg/L	149 (92.5–393)	38.4 (15.6–156)	13.0	−3.504	**<0.001**
Postdose t_{max}, h	3.0 (0.5–26.0)	3.0 (1.1–3.1)	65.0	−0.630	0.544
t_{last}, h	>30	>30 (8–>30)	42.0	−1.988	0.078
$AUC_{0–30}$	2630 (1712–9134)	481 (52.8–1719)	1.0	−4.161	**<0.001**
$AUC_{>baseline}$	274 (1.8–1294)	481 (47.3–1334)	55.0	−1.2	0.244

[a] Values in bold are statistically significant.

quent smokers receiving oral THC developed tolerance and required significantly more smoked cannabis to achieve similar levels of "high," supporting our hypothesis that frequent smokers may inhale significantly more cannabis to achieve a desired effect. Furthermore, others documented that a longer history of frequent cannabis intake correlated with total volume of cannabis smoke inhaled per cigarette, higher average smoke volume per puff, longer average puff duration, and faster puff velocity *(21)*. In our study, participants smoked ad libitum, possibly leading to even greater interindividual differences in smoking topography.

THCCOOH and THCCOO-glucuronide were significantly higher in frequent smokers' blood and plasma at all time points. There was no overlap in THCCOOH blood and plasma concentrations between frequent and occasional smokers, except at 0.5 h. Although 1- and 6-h THCCOO-glucuronide blood and plasma concentrations were similar to those reported by Schwope et al. *(7)*, THCCOOH blood and plasma concentrations in our frequent smokers were higher [6-h blood median of 23.9 μg/L (16.2–77.8 μg/L) compared with 16 μg/L (6.4–39 μg/L) and 6-h plasma median of 47.7 μg/L (25.7–114 μg/L) compared with 26 μg/L (9.6–61 μg/L)]. We postulate that this is because the participants in that study had a longer time since last cannabis consumption [median of 2.0 days (1–4 days)] compared with our participants [median of 4.1 h (1.2–23.2 h) from time of admission, or 1 day (1–2 days)].

Because of the extended excretion of THCCOOH and THCCOO-glucuronide, the group t_{last} could not be determined or statistically compared except for THCCOO-glucuronide in blood, which was earlier in occasional smokers. If THCCOOH and THCCOO-glucuronide were monitored beyond 30 h, we postulate a significantly earlier t_{last} for occasional smokers, given the extended excretion of THCCOOH in abstinent chronic frequent smokers *(11)*. It should be noted that the earlier t_{last} values observed for THCCOO-glucuronide compared with THCCOOH in this study are likely a consequence of THCCOO-glucuronide's higher LOQ (5 μg/L vs 1 μg/L for THCCOOH). Kelly and Jones *(8)* documented unconjugated and conjugated THCCOOH before and after alkaline hydrolysis and reported shorter detection windows in occasional smokers compared with frequent smokers for unconjugated THCCOOH; however, they administered intravenous THC, had only 4 participants in each group, and did not statistically evaluate differences. Furthermore, baseline THCCOOH concentrations in the frequent smokers were at least 2-fold lower than in our frequent smokers. Others have also documented higher concentrations *(9, 12)* and longer detection windows for THCCOOH and THCCOO-glucuronide in frequent smokers, although in 1 study no statistical comparisons were undertaken *(9)*.

THCCOOH and THCCOO-glucuronide $AUC_{0–30}$ were significantly higher (without correcting for baseline concentrations) in frequent smokers in plasma and blood in our study; plasma THCCOOH $AUC_{>baseline}$ was higher in occasional smokers. This contrasts with time-corrected overall THCCOOH and THCCOO-glucuronide blood and plasma concentrations, which were higher in frequent smokers with and without baseline correction. Toennes et al. *(12)* also documented significantly higher AUC and C_{max} in frequent smokers following paced smoking, suggesting that these differences were due to previous cannabis use, as C_{max} and AUC were no longer significantly different after adjusting for initial THCCOOH concentration. We hypothesize that the lack of consistency between our 2 methods to evaluate overall group effects ($AUC_{>baseline}$ and general mixed linear model) is explained by the extremely high baseline concentrations in frequent smokers; median plasma baseline THCCOO-glucuronide concentration was 103 μg/L, compared to not detectable in occasional smokers. In frequent smokers, median THCCOO-glucuronide concentration declined below their base-

line concentrations by 6 h, whereas occasional smokers were still above their baseline (not detectable concentrations) after 30 h. Therefore, the $AUC_{>baseline}$ method may be too severe a way to account for baseline concentrations, to the point where it overcompensated (and occasional smokers' $AUC_{>baseline}$ actually became significantly higher than the frequent smokers' in some cases). The general linear mixed model method to compare group concentrations may be a better approach than the $AUC_{>baseline}$ approach that others previously used *(12)*.

THCCOOH was detected more frequently and for a longer period of time than THCCOO-glucuronide. Because THCCOO-glucuronide concentrations were almost always higher than THCCOOH concentrations, the lower detection rates and earlier t_{last} values observed in this study likely result from the higher LOQ for THCCOO-glucuronide. In fact, previous work quantifying THCCOOH before and after hydrolysis documented higher concentrations and longer detection windows for the conjugated THCCOOH in 7 of 8 participants (THCCOOH LOQ of 1 μg/L) *(8)*. High variability in THCCOO-glucuronide:THCCOOH ratios precluded their ability to predict recent use.

CBD, CBN, and THC-glucuronide were detected for much shorter times than THC, THCCOOH, 11-OH-THC, and THCCOO-glucuronide. Blood and plasma CBN and plasma CBD C_{max} were significantly higher in frequent smokers (although individual time point concentrations were not significantly different because of the Bonferroni correction). The fact that C_{max} concentrations were significantly higher in frequent smokers (when CBD and CBN baseline blood and plasma were negative) further supports our hypothesis that frequent smokers inhaled substantially more cannabis than occasional smokers. Although CBD and CBN have similar contents in the cannabis cigarette (0.25% and 0.21%, respectively), CBN had a higher detection rate and concentrations than CBD. It is unclear why such differences exist, but differences in analyte absorption and distribution are not suspected because CBD and CBN have similar systemic availability following smoking [31% (13%) and 39% (26%), respectively], and CBD has a lower volume of distribution compared to CBN [32.7 (8.6) L/kg and 50 (23) L/kg, respectively] *(22, 23)*. We hypothesize that CBN concentrations may increase during the smoking process, given that THC can decompose to CBN *(24)*. Similarly, much higher CBN concentrations relative to CBD were reported in cannabis smoke compared with cannabis vapor *(25)*.

CBD, CBN, and THC-glucuronide can be used as inclusionary markers of recent use, as previously suggested *(7)*. Our cannabis cigarettes had a low CBD concentration [0.25% (0.08%)]; higher CBD concentration strains could lead to longer CBD detection windows.

In summary, we performed simultaneous THC, 11-OH-THC, THCCOOH, CBD, CBN, THC-glucuronide, and THCCOO-glucuronide analysis in blood and plasma from frequent and occasional smokers following controlled cannabis smoking. THC, 11-OH-THC, THCCOOH, and THCCOO-glucuronide were significantly higher in frequent smokers compared with occasional smokers, even after correcting for baseline concentrations. CBD, CBN, and THC-glucuronide could be used as inclusionary markers of recent use. These data will improve blood and plasma cannabinoid interpretation in DUID (driving under the influence of drugs) and accident responsibility cases.

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